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Goater, Cameron P

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POPULATION BIOLOGY OF *MEIOGYMNOPHALLUS MINUTUS* (TREMATODA: GYMNOPHALLIDAE) IN COCKLES FROM THE EXE ESTUARY

CAMERON P. GOATER

Department of Biological Sciences, Hatherly Laboratories, University of Exeter, Exeter, EX4 4PS
Present address: Zoologisches Museum der Universität Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

Spatial, seasonal and age-related differences in infection of the cockle, *Cerastoderma edule*, with the trematode *Meiogymnophallus minutus* were studied on the Exe Estuary, England. Prevalence of infection was 100% across all samples with mean abundances between approximately 300 and 1000 larvae per host (maximum=4930 larvae). Aggregration of *M. minutus* in cockles was extremely high (variance:mean ratios >100) and increased linearly as abundance increased. Mean abundance was highest in cockles collected from muddy substrates where the average age of cockles was high. In summer, numbers of larvae fell to less than half of spring levels and then increased after infection by a new wave of cercariae in August. Cockles accumulated larvae for up to 2 y but not thereafter, and levels of aggregation fell in the oldest age class. A peaked pattern of mean abundance and aggregation with age may be a result of parasite-induced death of older hosts or may be due to the inability of cercariae to establish in older hosts.

The seasonal drop in infection levels in summer, and the general stability of infection levels with age may also be due to mortality of the parasite induced by the pathogenic sporozoan *Unikaryon legeri*. Infected metacercariae were found in 85% of the total sample of cockles but were particularly common in heavily infected, older cockles from muddy substrates. Mortality of *M. minutus* due to hyperparasitism was highest in July, when up to 75% of larvae from cockles collected at one site were dead.

INTRODUCTION

Predation, competition and environmental disturbance have traditionally dominated studies which aim to determine the distribution and abundance of marine macroinvertebrates, especially molluscs (review in Underwood & Denley, 1984). The importance of parasite infection has largely been ignored, despite the accumulating evidence that parasitism may both directly and indirectly affect the ecology of their hosts (review in Anderson, 1989; Minchella & Scott, 1991) and that some marine hosts, especially shellfish, are among the most heavily infected hosts yet studied (review in Lauckner, 1980; 1983; Rhode, 1982).

While the importance of trematode infection (usually castration) to marine molluscs has been recognized at the level of individual snails as first intermediate hosts (Lauckner, 1980; Sousa, 1983), there are very few studies which examine the importance of larval

infection in molluscs which are used as second intermediate hosts. Trematode larvae in second intermediate hosts are known as metacercariae, and they usually encyst within species-specific regions of the host; in molluscs this is usually within the digestive gland, foot or mantle (Lauckner, 1983).

Cockles (*Cerastoderma* (= *Cardium*) *edule* (L.)) throughout Europe are hosts for the larvae of up to ten species of trematode and an individual can be infected by a total of up to 10,000 larvae (Lauckner, 1983; Goater, 1990). The cockles act as second intermediate host to parasites which mature in waterbirds, especially gulls (*Larus* spp.) (see Goater, 1990). In this paper I examine patterns of infection of cockles with *Meiogymnophallus minutus* Cobbald collected on the Exe Estuary, Devon. It differs from the typical digenean life-cycle in that it uses a bivalve for both first and second intermediate host (*Scrobicularia plana*(da Costa). and *C. edule* respectively). Metacercariae are small (0·12-0·30 mm long) and are located under the hinge-line of cockles in estuarine and sheltered habitats (Bowers & James, 1967). It uses waterbirds, especially oystercatchers, as final host (Goater, 1990). An additional feature of this host-parasite system is that metacercariae are often infected with a pathogenic sporozoan parasite, *Unikaryon legeri* Dollfus (Canning & Nichols, 1974; Lauckner, 1983). Infected metacercariae can easily be distinguished from uninfected ones so it is possible to quantify the effects of hyperinfection on the overall population dynamics of this parasite. Specifically, this study examines the spatial, seasonal and age-related patterns of *M. minutus* infection in cockles and asks whether characteristics of infection on the estuary are indicative of parasite-induced host mortality (*e.g.* Anderson & Gordon, 1982) and whether events occurring within the second intermediate host are important to the overall population dynamics of this parasite.

MATERIALS AND METHODS

Study area

The study area (Figure 1) includes the estuary of the River Exe in southern England (50°45′N 3°30′W). It is a small estuary which contains large populations of non-breeding wading birds and wildfowl which are primarily dependent on the extensive populations of mussels, cockles and other macro-invertebrates for food. Boalch (1980) reviews the general abiotic and faunistic characteristics of the Exe Estuary and Goss-Custard *et al.* (1992) and McGrorty & Goss-Custard (1991) review the general characteristics of the well-studied oystercatcher and mussel populations, respectively. There is one extensive, commercial cockle bed, known as Cockle Sands (Bed 6, Figure 1) where cockles reach densities comparable to other commercially important sites around Britain, but elsewhere on the estuary they exist at much lower densities (see below).

Field collections - cockles

To examine spatial patterns of infection in cockles, six sites were chosen to reflect a continuum of abiotic and biotic conditions (Figure 1). Four sites were mussel beds which had previously been studied by Goss-Custard *et al.* (1982, 1992) and McGrortry & Goss-

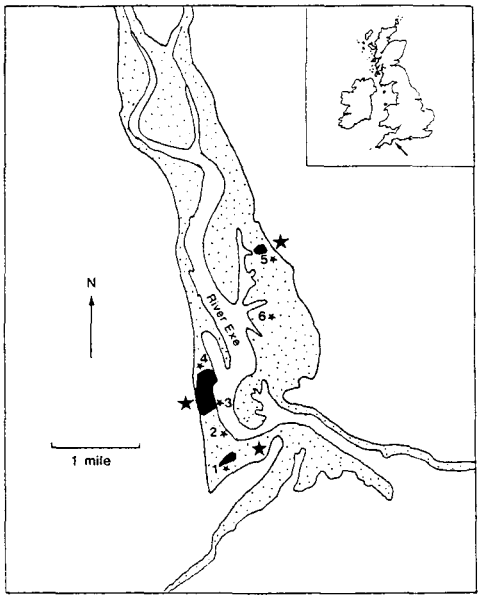

Figure 1. The Exe Estuary and its major substrate types. Numbered stars indicate cockle beds which were analysed for infection with *Meiogymnophallus minutus*. Large stars indicate locations where oystercatchers were collected. Black areas, mussel beds; stippled, mud and sand exposed at low water.

Custard (1991). The six beds were fenced to 50 m^2 with transect rope to provide distinct and uniform sampling areas. In November 1988, 25 random samples were collected on each bed using a 0·25 m^2 quadrat on the five low-density beds and 0·1 m^2 quadrat on Cockle Sands. Cockles comprising each sample were counted and then separated into individual age cohorts as determined by the numbers of annual growth rings (Richardson *et al.*, 1980). Cockles were sorted into age classes and between 30 and 50 cockles from each class were measured to the nearest mm (maximum length from umbo). These field measurements provided comparative estimates of host density, age structure and size structure across the six beds. From these collections 25 two-year-old cockles were selected at random and brought to the laboratory for analysis of infection with *Meiogymnophallus minutus* larvae. This cohort was selected because of its generally high abundance throughout the estuary and because at other localities oystercatchers are known to prefer this size class (Drinnan, 1957; Davidson 1967).

I also measured differences in substrate composition between the six beds because this may influence the transmission of cercariae. Comparative estimates of substrate composition were measured following a technique adapted from Buchanan (1984). Vials (2x6 cm) were inverted and pushed into the substrate on ten random sites on each bed. The ten plugs of substrate were mixed thoroughly in a plastic bag, 50·0 g removed and then placed in a graduated cylinder. Water was added up to 100 ml, the solution was mixed and allowed to settle into fractions. Samples of substrate from each fraction were removed by pipette and measured under a compound microscope. All fractions of substrate with mean grain size greater than 0·125 mm (Buchanan, 1984) were added together and the summed value was used as a proportional estimate of sand concentra-

tion on each bed.

The seasonal pattern of larval infection was examined in four-year-old cockles collected from Bed 5 (Figure 1). Twenty-five cockles with three distinct growth rings were collected between the 28th and 31st of each month from April to September 1988 and the numbers of *M. minutus* were determined. Cockles from this bed were also dissected to examine the relationship betweeen host age and larval infection. Twenty-five cockles collected in May 1988 were examined from each of seven age classes.

Field collections - oystercatchers

Oystercatchers, the final host of *Meiogymnophallus minutus*, were also collected from the estuary as part of a larger study which examined the overall dynamics of helminth infection in oystercatchers and their shellfish intermediate hosts (Goater, 1990). Methods of collection and the detailed patterns of infection and life-cycles of all other species of helminth in oystercatchers are detailed in Goater (1990). For the purposes of this paper, I present summary infection characteristics of *M. minutus* from 60 oystercatchers collected from January 1986 to July 1988. These birds had been shot as part of a multidisciplinary study; intestines were flash frozen in the field (Bush & Holmes, 1986) and the intestine remained frozen until examination in the laboratory.

Parasite counts

Meiogymnophallus minutus metacercariae are located in a cavity beneath the hinge in a characteristic mass of white, chalky tissue. Metacercaria are unencysted but are enveloped by the extrapallial edge of the host mantle (Bowers & James, 1967). Metacercariae are often infected with a pathogenic hyperparasitic sporozoan, *Unikaryon legeri* (Lauckner, 1983; Canning & Nichols, 1974). Dead metacercariae can easily be distinguished from living: the former are distended to approximately double normal size and are white.

The large numbers of *M. minutus* and the difficulties imposed by the presence of dead and dying metacercariae made precise measurements of absolute abundance impossible. A dilution technique was therefore used to estimate abundance. The tissue below the umbo was removed and immersed in a solution of sea-water and invertebrate saline. This tissue was composed of tightly packed living metacercariae contained within plugs of host tissue, plus dead and dying metacercariae. There was often large amounts of dark, solid debris which could occupy the entire cavity below the hinge. This tissue was teased apart under a microscope (x10) leaving living and dead metacercariae free in solution. The solution was increased to 100 ml in a graduated cylinder and inverted four times. Two 10-ml aliquots were removed and numbers of living and dead metacercariae counted under a microscope. If the two samples differed by more than 15%, a further sample was taken. Counts were totalled and the number of metacercariae per cockle was estimated. Cockles were examined as quickly as possible (within 5 d) after collection to reduce the likelihood of *M. minutus* metacercariae dying between the time of collection and examination.

Mortality of *M. minutus* was estimated by the numbers of dead metacercariae in

individual cockles. Such estimates were based on the assumption that all dead metacercariae were killed by hyperparasitic infection. Other mortality factors and natural senescence may have occurred but were assumed, for purposes here, to be less important than that induced by hyperinfection. It must be noted that, in some cockles, a large amount of pearl-like debris was associated with metacercariae. This was undoubtedly composed of parasites in varying degrees of decomposition. In these hosts the distinction between 'dead' metacercariae and this debris was often unclear, in which case, mortality may be underestimated.

Adult *M. minutus* are located throughout the length of the small intestine of oystercatchers, often in numbers greater than 1000 worms per host. A dilution technique, similar to the one noted above for larval counts in cockles, was used to estimate adult parasite numbers. These estimates were made on the numbers of adult parasites within sections of intestine, each of which represented 5% of the total intestinal length (see Goater, 1990). Each of the 20 sections was examined individually and the total number of *M. minutus* per bird was determined.

Analysis

Estimates of the numbers of living and dead metacercariae were overdispersed and non-normally distributed within samples of cockles. Larval counts were therefore log-transformed to minimize the heterogeneity of variances among samples. Differences between samples were tested with a one-way ANCOVA with (log) host size as the covariate. Comparisons between means used Scheffe's multiple range procedure. The survival of larval *Meiogymnophallus minutus* was estimated as the proportion of total larvae which were living at the time of dissection, arcsine (square root) transformed before analysis. Throughout this paper I follow the recommendations of Margolis *et al.* (1982) in defining mean abundance as the average number of parasites per host and mean intensity as the mean number of parasites per infected host. Suprapopulation refers to the total population of parasites within all sampled hosts. To analyse the dispersion of parasites within cockle samples I used the ratio of the variance in parasite counts to the mean. This index is more useful than the familiar k of the negative binomial distribution when interest is focused on comparing parasite dispersion patterns between samples (Scott, 1987).

RESULTS

The cockle population

The density of cockles on the Exe Estuary, with the exception of Bed 6 (Table1; see also Goater, 1990), is characteristic of other sites around Britain where conditions for settlement and growth are poor (Boyden & Russell, 1972; Sanchez-Salazar *et al.*, 1987). On Bed 6 the density, average age and average size of cockles are consistent with other studies where cockles were collected from sandy substrates (O'Conner & Brown, 1977; Sutherland, 1982; Boyden, 1972). In general there was a significant negative correlation between

Table 1. *Summary data (mean ±SD) for selected site characteristics of six cockle beds on the Exe Estuary*

Bed	% sand	Density	Host age	Host size	Size at age 2 y
1	53.5	35.8 ±22.8	3.3 ±1.5	25.3 ±4.1	23.7 ±1.5
2	59.3	11.2 ±8.2	3.0 ±1.5	30.6 ±4.3	29.2 ±3.2
3	76.0	22.8 ±14.6	2.8 ±1.5	27.4 ±6.6	27.2 ±1.7
4	66.7	15.2 ±6.3	2.7 ±1.5	29.3 ±6.4	28.3 ±2.2
5	61.9	13.2 ±10.9	3.7 ±1.7	25.7 ±6.0	23.2 ±2.2
6	94.7	160.9 ±81.3	2.1 ±1.3	20.0 ±5.7	20.2 ±1.2

the mean size of cockles on the six beds and their density (N=6, r=-0·923, P<0·05). In addition the mean size of cockles on the beds was not correlated with their average age (N=6, r=-0·483, P>0·05) but was correlated with their average size at 2-y of age (N=6, r=-0·954, P<0·05). These results suggest that differences in the mean size of cockles on the beds were determined by differences in their rates of growth and not differences in their ages. Differences in growth rate probably also best explain differences in the average sizes of 2-y-old cockles on the beds ($F_{5,144}$=76·66, P<0·001). In descriptive terms the single sandy bed was associated with cockles of high density but low average age and size. The remaining 'muddy' beds were generally composed of cockles at low density, which on average were older and larger due to their relatively high growth rates.

General infection patterns in cockles

All cockles except newly-settled spat were infected with *Meiogymnophallus minutus*. Eighty-five percent of the 150 cockles sampled also contained metacercariae which were infected with *Unikaryon legeri*. Estimates of the numbers of dead metacercariae were strongly associated with the numbers of living (y=0·56x + 0·68, df=448; R^2=0·040; P<0·001). Intensities of infection were generally high and extremely variable both within and across cockle samples. The distribution of living metacercariae within samples was extremely overdispersed with the variance of parasite counts always much greater than one and frequently greater than 100. In general the log of the variance of parasite counts increased linearly as log mean abundance increased (data from 17 combined samples; y=1·15 +1·38x, R^2=0·51; P=0·001). This result indicates that at least half the variance in parasite counts across samples could be explained by differences in mean abundance.

Spatial pattern of infection in two-year-old cockles

The prevalence of infection of *Meiogymnophallus minutus* in 2-y old cockles was 100% on all beds. Mean abundances of living metacercariae were significantly different across the six beds (Tables 2 & 3) increasing from approximately 500 metacercariae per host on Bed 6 to approximately 1500 metacercariae per host on Bed 1. Scheffe groupings showed that mean abundance on Bed 1 was significantly higher than on all other beds and that Bed 6 had lower mean abundance than all other beds except Bed 4.

Over 85% of the 150 cockles from the six beds contained metacercariae which were infected with *Unikaryon legeri*. The mean number of dead metacercariae, which was used

Table 2. *Infection characteristics of* Meiogymnophallus minutus *(mean ±SD) in 25 two-year-old cockles collected from six sites on the Exe Estuary*

	Bed						Total
	1	2	3	4	5	6	(N=150)
Prevalence	100	100	100	100	100	100	100
Mean abundance	1559±810	1012±319	952±322	720±260	1039±313	570±213	978±532
Variance/mean	421	100	109	94	95	80	298
Range	610-4930	165-1605	455-1670	326-1435	560-1835	250-1282	165-4930
Mean numbers dead	366±397	84±86	262±190	120±87	485±328	42±90	234±283
% mortality	19.0	7.6	21.6	14.2	31.8	6.8	19.3

Table 3. *Summary of ANCOVA statistics for spatial, seasonal and age-related differences in infection characteristics of living* Meiogymnophallus minutus *in cockles from the Exe Estuary. Main effects were adjusted for the effect of the covariate (log cockle size). Data were log-transformed prior to analysis*

	Response variable	df	Mean square	*F*	*P*
Sites (N=6)					
	Numbers of larvae/host				
	Covariate	1	0.082	3.15	0.078
	Site	5	0.516	19.77	<0.001
	Residual	143	0.026		
	Total	149	0.043		
	Numbers of dead larvae/host				
	Covariate	1	9.248	18.08	<0.001
	Site	5	10.582	20.69	<0.001
	Residual	143	0.512		
	Total	149	0.908		
Months (N=6)					
	Numbers of larvae/host				
	Covariate	1	0.275	4.01	0.047
	Month	5	0.730	10.63	<0.001
	Residual	143	0.069		
	Total	149	0.092		
	Numbers of dead larvae/host				
	Covariate	1	0.005	0.03	0.874
	Month	5	1.435	7.85	<0.001
	Residual	143	0.183		
	Total	149	0.224		
Age*					
	Numbers of larvae/host				
	Covariate	1	0.985	8.52	0.004
	Age	5	0.604	5.28	<0.001
	Residual	143	0.116		
	Total	149	0.138		
	Numbers of dead larvae/host				
	Covariate	1	95.202	397.40	<0.001
	Age	5	2.439	10.18	<0.001
	Residual	143	0.240		
	Total	149	0.951		

*analysis does not include uninfected spat.

as an indicator of the extent to which they were hyperparasitized, was significantly different across the six beds (Table 3). Metacercariae infrapopulations in cockles from Beds 5 and 1 were the most heavily infected; Beds 6 and 2 were the least (Table 2). The proportion of living metacercariae within infrapopulations was also significantly different across the beds ($F_{5,149}$=14·28, P<0·001); on Bed 5 over 30% of total metacercariae were dead while less than 7% were dead on Bed 6.

I examined for associations between infection patterns on the six beds (Table 1) and the selected abiotic and biotic characteristics of the beds (Table 2). There was a positive, but non-significant, association between the numbers of living metacercariae and the mean age of cockles (N=6, r=0·808, P<0.10) and a negative association between the numbers of metacercariae and the sandiness of the beds (N=6, r=-0·827, P<0·05). Also, the abundance of dead *M. minutus* was significantly correlated with the mean age of cockles on the beds (N=6, r=0·868, P<0·05). Heavy infections with living *M. minutus* were therefore associated with cockles collected from relatively muddy beds where conditions for growth were poor and the mean age of cockles was high. On these same beds there was an increase in the numbers of metacercariae which were infected with *U. legeri*.

Temporal patterns of infection

Prevalence of *Meiogymnophallus minutus* infection remained at 100% on Bed 5 over the six months. There were significant monthly changes in the mean numbers of living

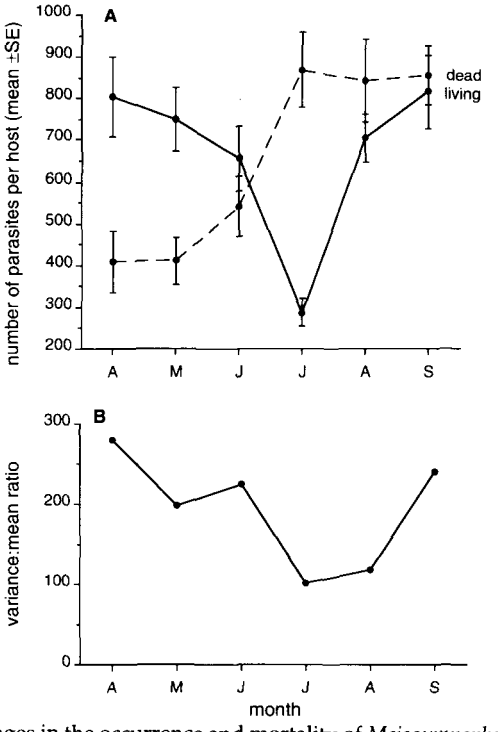

Figure 2. Seasonal changes in the occurrence and mortality of *Meiogymnophallus minutus* found in 25 4-y-old cockles from Bed 5: (A) changes in mean abundance of living and dead metacercariae; (B) changes in the degree of overdispersion of living metacercariae.

metacercariae with a sharp decline from April to July, followed by a sharp increase (Table 3; Figure 2A). Scheffe groupings showed that the decrease from June to July was significant but that mean abundance increased after July to levels unchanged from the previous spring. The mean numbers of dead metacercariae in cockles also showed a distinct pattern of seasonal change (Table 3, Figure 2A). Numbers of dead *M. minutus* rose sharply to reach a maximum in July when 75% of the total numbers of metacercariae were dead, and then remained constant from July to September. The large increase in the total numbers of living metacercariae after July, without a concomitant increase in the numbers of dead ones, provides evidence for the immigration of new cercariae from *Scrobicularia plana* into the cockle population during this month.

Immigration and mortality processes interacted to determine the complex seasonal pattern of dispersion for *M. minutus* (Figure 2B). The decline in $s^2/\bar{x}$ to a minimum in July, mirroring the period of maximum mortality, but prior to new immigration, showed that *U. legeri* reduced the mean numbers (and variance) of metacercariae (Figure 2B). However, the sharp rise in mean abundance in August without a concomitant rise in the $s^2/\bar{x}$ implied a relatively uniform and homogeneous period of infection (*sensu* Anderson & Gordon, 1982). In September the $s^2/\bar{x}$ rose back to spring levels, possibly as a result of increased variation due to mortality induced by hyperparasitic infection.

Age-related patterns of infection

All cockles over 1-y old (one or more rings) were infected with *Meiogymnophallus*

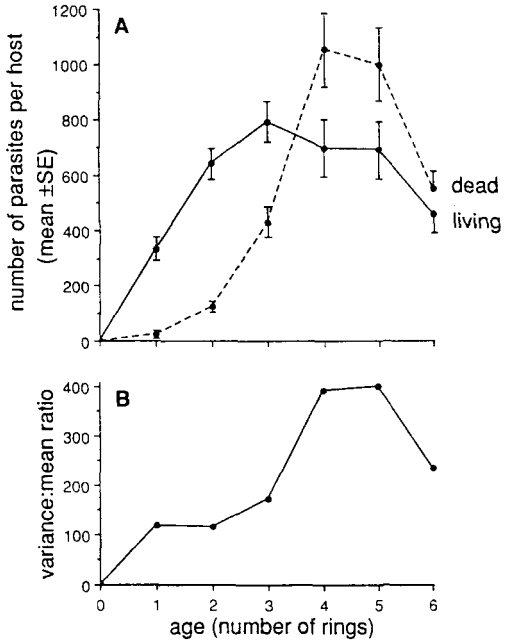

Figure 3. Changes in the occurrence and mortality of *Meiogymnophallus minutus* with age in cockles collected from Bed 5: (A) changes in mean abundance of living and dead metacercariae; (B) changes in the degree of overdispersion of living metacercariae.

minutus (Figure 3A). There were highly significant differences between age classes in mean abundance (Table 3). Scheffe groupings showed that there was a significant increase in numbers of larvae between 1- and 2-y-old cockles, after which a plateau in larval numbers was reached between 3- and 6-y-olds. The decline in larval counts between 5- and 6-y-olds was not significant.

There were also highly significant differences between age classes in estimates of the mean abundance of *M. minutus* killed by *Unikaryon legeri* (Table 3). Numbers of dead metacercariae rose sharply with age, reaching a maximum in 4-y-olds and remaining unchanged to age six (Figure 3A). Scheffe groupings showed that the increase in numbers of dead larvae was significant between 1- and 2-y-olds and then remained unchanged between ages three and six. The decline in mean numbers of dead metacercaria in 5- and 6-y-olds was not significant.

The distribution of living *M. minutus* was extremely aggregated ($s^2/\bar{x}$ >100) in all infected age groups (Figure 3B). The degree of aggregation rose to a peak in 4-y and 5-y hosts and declined sharply in the oldest age class. Since the mean number of living metacercariae remained constant (statistically) in the older age classes, the decline in $s^2/\bar{x}$ was associated with decreasing variance in parasite counts.

Patterns of infection in oystercatchers

The numbers of *Meiogymnophallus minutus* in oystercatchers was extremely variable with the standard deviation of parasite counts always much higher than the mean (Table 4). Overall, 32 of 60 birds were infected with from 1 to over 22,000 worms. Such high parasite burdens are characteristic of other gymnophallid and microphallid trematode infections, especially in aquatic birds (*e.g.* Bush, 1990).

Table 4. *Patterns of infection of* Meiogymnophallus minutus *in oystercatchers from the Exe Estuary*

	Autumn	Winter	Spring	Summer*
N	10	31	7	11
Prevalence (%)	60.0	51.6	57.1	54.5
Mean abundance (±SD	515±1033	2040±5199	49±108	1395±4623
Mean intensity (±SD)	857±1252	3956±6781	86±139	2557±6259
Range	0-2957	0-22594	0-293	0-15533

* infection data for birds collected in summer are greatly affected by a heavy infection of 15,333 worms in one juvenile. Omitting this bird results in mean abundance levels falling to 1.1±2.1 worms

The seasonal pattern of infection (Table 4; see also Goater, 1990) shows that juvenile birds become infected almost immediately upon arrival on the estuary. In winter, 5 of 31 birds had over 5000 worms. This small proportion of birds may represent the small proportion on the Exe which selectively feed on cockles during winter (Goss-Custard & Durell, 1983). In spring and summer, when most birds are juveniles and feed on mussels, all but one bird had less than 300 worms. However, the presence of one heavily infected bird is important because it demonstrates that larvae are still present, and infective, during the time when mortality of larvae is at a maximum (Figure 3).

DISCUSSION

The general pattern of infection of cockles with *Meiogymnophallus minutus* is characteristic of other host-parasite systems involving larval trematodes in their second intermediate hosts: high prevalence, high spatial variability, temporal variability corresponding to the release of cercariae in spring or summer and a general increase in abundance with the age or size of the host (*e.g.* Lemly & Esch, 1984; Spelling & Young, 1986; Kennedy, 1984, 1985; Ménard & Scott, 1987). The pattern of dispersion of *M. minutus* within cockle samples is also characteristic of other larval host-parasite systems, showing high overdispersion with the level of parasite aggregration generally associated with an increase in mean abundance. However, two unique aspects of *M. minutus* infection in cockles are the very high intensities of infection (up to approximately 5000 larvae per host) and their association with the pathogenic sporozoan, *Unikaryon legeri*. Very little is known about the biology of hyperparasitic infection in metacercariae, but it is clear that hyperparasitism greatly affects the overall pattern of infection of *M. minutus* in cockles.

The association between a muddy substrate and the numbers of *M. minutus* may best explain why levels of infection of cockles on the Exe are much higher than those reported elsewhere in Europe (Bowers & James, 1967; James *et al.*, 1977; Russell-Pinto, 1990). Only one of six sites studied by James *et al.* (1977) had intensities of infection (mean=1240 larvae/cockle) comparable to those found on the Exe. In this study, cockles from the least infected site had intensities of infection in 2-y-olds at least double those reported from cockles in the Burry Inlet (Bowers & James, 1967) and the coast of Portugal (Russell-Pinto, 1990). One explanation for the association between substrate and infection, as suggested by James *et al.* (1977) and Russell-Pinto (1990), lies in the preference of the first intermediate host, *Scrobicularia plana*, for muddy substrates. Bed 5, for example, where cockles had high intensities of infection (Table 2), has one of the highest concentrations of *S. plana* on the estuary, and also has relatively large numbers of oystercatchers selectively feeding on clams and cockles (Boates & Goss-Custard, 1989). The close association of all three hosts required in the life cycle may best explain such high intensity infections, as has been suggested for other marine host-parasite systems (Carrol *et al.*, 1990; Copeland *et al.*, 1987; Curtis & Hurd, 1983; Irwin & Irwin, 1980). An alternative (but not necessarily independent) explanation is that infection characteristics of trematode larvae on the estuary result from the strength and direction of tidal currents. Goater (1990) suggested that infection patterns of non-motile cestode larvae in cockles were determined by local conditions on each bed (especially bird and intermediate host density), whereas infection with motile trematode larvae was more dependent on estuary-wide conditions, with specific sites on the estuary acting as 'sinks' for larval infection. Such a mechanism may play a unique and important role in estuarine and coastal host-parasite systems for it implies that factors which determine specific settlement sites will play an important role in determining an individual's ultimate exposure to parasite larvae.

The association between intensity of infection and the age of cockles on the beds may also be related to the muddy conditions on the estuary and the generally poor conditions for growth on most beds. Other workers have shown that cockles from muddy substrates

are generally old and exist at low population densities (Boyden & Russell, 1972; Boyden, 1972). Most simply, older cockles will have experienced more annual waves of infection in summer and will have accumulated more larvae. These older cockles on muddy substrates will also experience lower levels of predation due to their larger size and low density (Sutherland, 1982) and the relatively low numbers of oystercatchers feeding on muddy substrates (Goss-Custard & Durell, 1983). Beds with relatively low infection levels such as Bed 6, have cockles which, on average, are relatively young and small and experience higher levels of predation. The results from this study suggest that one consequence of settlement in muddy areas is increased levels of infection by *M. minutus* and probably other helminth larvae (Goater, 1990). The consequences of relatively high levels of infection to cockles, especially those on muddy substrates, are, however, unknown and require specific experimentation.

The extremely variable patterns of infection in oystercatchers can best be explained by variation in host diet. In observations of individually marked birds, Goss-Custard & Durell (1983) showed that while most birds preferred and specialized on mussels (determined by repeated observations of individual birds over several years), less than 5% specialized on cockles. Infection data presented here support their observations if it is assumed that only those birds which had the heaviest infections were cockle specialists. The overall drop in levels of infection in summer can also be explained by host diet. Juveniles were collected directly off mussel beds and, as shown by Goss-Custard & Durell (1983), these birds feed almost exclusively on mussels. However, it is possible that juvenile birds in summer, despite their relatively low numbers and their low levels of infection, may be important to the overall transmission of *M. minutus* to first intermediate hosts, and subsequently to cockles. Goater (1990) showed that transmission of the trematode *Psilostomum brevicolle* Creplin to cockles on the estuary was primarily due to the small proportion of juvenile oystercatchers which remained on the estuary during summer.

A plateau in the average numbers of parasites with host age, concomitant with a peak in the pattern of overdispersion, was suggested by Anderson & Gordon (1982) as a possible indicator of parasite-induced host mortality. They predicted that the death of old, heavily infected hosts could result in decreased mean abundance and a large decrease in the variance of parasite counts within older age classes. Such an interpretation is plausible in this system if cockles older than five years (Figure 3) suffer disproportionately from infection. This could occur directly, if infection affects survival of the oldest cockles, or indirectly if, for example, the accumulation of living and dead parasites under the hinge interferes with the operation of the valves. Bowers & James (1967) and Lauckner (1983) also speculated on the pathogenicity of *M. minutus* but they, as here, could not provide experimental evidence. In the absence of such data it is not possible to comment further on the possible role of infection on cockles, especially since peaked patterns of infection with respect to age can have alternative explanations (Kennedy, 1984). One is that there is a decline in the quality of older hosts which may influence cercarial immigration or survival. Older cockles accumulate dead metacercariae (Figure 3), possibly due to the ingestion of more spores of *U. legeri*. In many cockles a large

amount of debris, which is composed of dead metacercariae, can occupy the entire space below the umbo. Thus one possibility for the pattern of decreased larval abundance and dispersion in older cockles may be the inability of cercariae to penetrate this debris and/or the decreased space available under the umbo in older cockles.

In addition to the probable influence of *U. legeri* on the accumulation of parasite larvae with age, hyperparasitism strongly influences other aspects of *M. minutus* infection in cockles. In this study the absolute numbers of dead metacercariae were used as a crude index of mortality induced by hyperparasitism, a method which is almost certainly prone to error. In general, however, hyperinfection appeared, as for *M. minutus*, to vary spatially, seasonally and with respect to host age. Mortality induced by hyperinfection was highest on beds where cockles were heavily infected by *M. minutus* and thus where cockles were, on average, old and large (Table 2). Two mechanisms may explain this result. First, factors which influence the favourable transmission of cercariae from *S. plana* to cockles may also favour the transmission of spores of *U. legeri* to cockles (*e.g.* tidal currents or some other factor related to a muddy substrate), particularly if cercariae can become infected prior to ingestion by cockles. Second, the numbers of hyper-infected larvae will be strongly related to the numbers of *M. minutus* if the rate at which co-occurring metacercariae are infected is density-dependent. The increase in larval mortality in summer may be explained by either an increase in the rate of spore ingestion or an increase in the rate at which spores from infected metacercariae infect neighbouring metacercariae.

Regardless of the exact mechanism involved, hyperinfection clearly affects the seasonal pattern of *M. minutus* infection in cockles (Figure 2) and also affects the degree to which cockles accumulate metacercariae with age (Figure 3). Presumably such high larval mortality will affect the availability of infective larvae to final hosts and the subsequent transmission of the parasite through the system as a whole. These data, together with the possibility that *M. minutus* infection may cause host mortality (but see above), provide evidence to suggest that regulatory mechanisms within a second intermediate host may play an important role in the overall regulation of a parasite with a complex life-cycle. Whether the potential importance of infection to cockles is realized at sites other than the Exe, where intensities of infection are lower, or where the frequency of hyperinfection is lower, remains to be studied. More specifically, experimental laboratory studies are required which concentrate on the effects of *M. minutus* and its hyperparasite (in isolation from other larval trematodes) on individual hosts.

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